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Layer-dependent BOLD and CBV-weighted fMRI responses in the rat olfactory bulb

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ABSTRACT

The olfactory bulb is a laminarized brain structure involved in odor sensation that has important implications to basic neuroscience research, like mechanisms for neurovascular coupling and early disease diagnosis. To investigate laminar-dependent responses to odor exposure, blood oxygenation level-dependent (BOLD) and cerebral blood volume weighted (CBVw) fMRI with iron oxide nanoparticle contrast agent were obtained with $110 \times 110 \times 500\ \mu m^3$ resolution in urethane-anesthetized rats at 9.4 T. The baseline total CBV is the largest at the olfactory bulb surface and midline, and decreases in the deeper layers, while a band of increased microvasculature density is observed at the glomerular, external plexiform and mitral cell layers. With odor exposure, CBVw fMRI is more sensitive and reproducible than BOLD. BOLD fMRI had the greatest activation on the bulb surface, midline, olfactory nerve and glomerular layers, while CBVw activation peaked in glomerular and external plexiform layers, but was still significant in mitral cell layer. Negative BOLD responses were observed in the bulb midline and near large blood vessels. CBVw laminar profiles are similar to the layer-dependent metabolic changes to the same odor exposure reported by previous glucose metabolism studies. Unique activation patterns for two different odor conditions were also differentiated with CBVw fMRI. Our study suggests that CBVw activation better represents the spatial location of metabolic activity in the olfactory bulb than BOLD.

Introduction

The olfactory bulb is a unique model system beneficial for studying neurovascular coupling, learning and plasticity, and the early diagnosis of neurological disorders, which is anatomically and functionally organized by the thin bulb layers (Lancet et al., 1982; Sharp et al., 1977; Shepherd, 1972). The primary odor pathway in the bulb begins with the olfactory receptor neuron axons propagating through the olfactory nerve layer (ONL) to form dense excitatory synapses with the apical dendrites of mitral cells in the glomerular layer (GL). These dendrites transmit through the external plexiform layer (EPL) to the mitral cell body layer (MCL) and mitral cell axons exit the bulb to cortical targets, such as the piriform cortex. In addition, inhibitory granule cells in the granule cell layer (GCL) receive centrifugal projections from the cortex and form dendro-dendritic synapses with mitral cells in EPL. In this way, different functional components of the olfactory neural circuit are anatomically organized to the different bulb layers, thus allowing the study of how distinct neuro-electrical events, such as excitatory or inhibitory processes, contribute to the hemodynamic response. The study of neurovascular mechanisms in the olfactory bulb is popular with optical imaging techniques (Chaigneau et al., 2003, 2007; Gurden et al., 2006), but such studies are limited to layers near the bulb surface. Next, enhancement of odor discrimination to learned odor stimuli was shown to be accompanied by increased synaptic connections in discrete olfactory bulb glomeruli (Jones et al., 2008). This discovery provides an interesting fMRI model to study neural plasticity in a primary sensory brain region that can be easily evoked by odor in an anesthetized animal model; contrary to studying plasticity in more traditional brain regions, like the hippocampus, which are more difficult to evoke in an fMRI experiment. Finally, olfactory dysfunction is an early symptom of many diseases, such as multiple sclerosis, Parkinson's disease and Alzheimer's disease, that precedes the development of the more debilitating symptoms; and a greater understanding of in vivo odor function with fMRI can benefit such diagnostic applications. Therefore, high-resolution layer-dependent functional imaging of the entire bulb is highly desirable.

Abbreviations: fMRI, functional magnetic resonance imaging; BOLD, blood oxygenation level-dependent; CBVw, cerebral blood volume weighted; MION, monocrystalline iron oxide nanoparticles; ONL, olfactory nerve layer; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; GCL, granule cell layer; 2-DG, [^{14}C]-2-deoxy-D-glucose; FLASH, fast low-angle shot; ROI, region of interest.

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Layer-dependent metabolic changes to odor stimulation were historically examined with [^{14}C]-2-deoxy-D-glucose (2-DG) methods (Johnson et al., 1998; Lancet et al., 1982; Sharp et al., 1977). These studies showed that single odorants increased cellular metabolism in localized glomeruli (foci) and activation of different glomerular populations formed spatial patterns in GL that are unique to the odorant. In addition, line profiles through the bulb layers showed that cell metabolism varied with bulb depth. For example, a profile through an activation focus revealed increasing metabolism in ONL that peaks in GL and gradually decreased through the deeper layers (Fig. 7E) (Sharp et al., 1977). However, 2-DG measurements are performed ex vivo and only allow examination of a single odorant for each animal. More recently, in vivo manganese-enhanced MRI measured odor-specific activation patterns in GL and MCL that are similar to 2-DG (Chuang et al., 2009, 2010; Pautler and Koretsky, 2002), but were still limited to the testing of one odorant per MRI measurement. Other in vivo methods like optical intrinsic signaling (Rubin and Katz, 1999; Uchida et al., 2000) and fMRI (Kida et al., 2002; Liu et al., 2004; Martin et al., 2007; Schafer et al., 2005, 2006; Xu et al., 2000, 2003; Yang et al., 1998) overcome these limitations but at the cost of measuring signal changes that originate from the vasculature; while only fMRI technology preserves measurement in the deeper bulb layers. Results from original BOLD fMRI studies, similar to 2-DG, measured focal activations that had odor-specific activation patterns and had the largest signal change in ONL and GL. However, unlike 2-DG, limited signal change was observed in EPL and little to no change in MCL and GCL (Kida et al., 2002; Xu et al., 2000; Yang et al., 1998). This discrepancy between the superficial and deeper bulb layers can be explained by i) the baseline CBV contribution to BOLD (Kim and Ogawa, 2012), ii) large signal contributions from pial vessels, whereby magnetic field gradients from pial vessels contribute to the fMRI response in GL near the bulb surface (Kim and Ogawa, 2012), and/or iii) a mismatch between the location of metabolism and the hemodynamic response. Thus, it is critical to understand whether the BOLD response is directly related to the laminar-dependent baseline CBV in the olfactory bulb, and whether hemodynamic-based fMRI measures change in MCL and GCL.

To examine afore-mentioned issues, we obtained BOLD and cerebral blood volume-weighted (CBVw) fMRI with $110 \times 110 \times 500$ μm^3 resolution and compared their sensitivity, reproducibility and spatial activation profiles across layers. The CBVw fMRI technique with injection of iron oxide nanoparticles was chosen for its decreased contributions from large blood vessel signal changes and enhanced sensitivity in capillaries proximal to metabolically active cells (Kim et al., 2013; Mandeville and Marota, 1999; Mandeville et al., 1998; Zhao et al., 2006). Baseline total blood and microvasculature enhanced volumes were also measured and compared to the layer-dependent BOLD and CBVw fMRI activation patterns. Finally, CBVw fMRI was used to measure the activation patterns for two odors to determine if unique stimuli can be functionally differentiated.

Materials and methods

Animal preparation and odor stimulation

Six male Sprague-Dawley rats (315–415 g) were studied with approval from the University of Pittsburgh Institutional Animal Care and Use Committee. Animals were induced with 5% and maintained with 2% isoflurane gas in medical air and O_2 gases during all surgery. The right femoral artery and vein were catheterized for physiological monitoring and administration of fluids, anesthetic and contrast agent, respectively. A 15-mm diameter section of scalp directly above the olfactory bulb was removed to increase the coil sensitivity and to prevent image wrapping artifacts for the 7×7-mm^2 field of view (FOV). A sub-cutaneous dose of carprofen (10 mg/kg) analgesia was administered before the anesthesia was switched from isoflurane to urethane (1.3 g/kg i.p. induction, followed by continuous 0.1 g/kg/h i.v. maintenance). The animals under urethane anesthesia were free breathing and did not require intubation. Mean blood pressure was monitored through the arterial line and maintained between 70 and 130 mmHg (MP150, BioPac Systems Inc., Goleta, CA). In addition, the animals' rectal temperature was maintained at 37 ± 1 °C using a warm water circulator and breathing rate was recorded with a pneumatic pillow sensor. A 0.9% saline and 5% dextrose supplemental fluid was administered intravenously at 2–3 ml/kg/h. A single bolus of 15 mg Fe/kg Feraheme (ferumoxytol, AMAG Pharmaceuticals, MA) was intravenously injected for CBV weighting after the BOLD fMRI studies.

Odor stimulation was performed by a home-built apparatus with TTL-controlled solenoid valves (EW-98302-02 and −22, Cole-Parmer, Vernon Hills, IL) and one-way check valves (EW-98553-00, Cole-Parmer) (Fig. 1A). The solenoid valves diverted airflow (1 L/min) to one of three 500 mL Pyrex flasks (EW-34503-07, Cole-Parmer) containing 5% amyl acetate in mineral oil, 1% pyridine or 100% mineral oil. Each odor condition had dedicated Tygon tubing (EW-95631-05, Cole-Parmer) with one-way check valves (indicated by arrowheads) to prevent mixing. The dedicated lines converged to a single line (~1 cm^3 common volume) before purging into a nose cone sealed around the rat snout. The dedicated lines allowed for quick transitions between odor conditions despite the long distance the odor had to travel from the odor source (~5 m). A vacuum line at the opposite end of the nose cone removed the odors. This odor stimulation system was synchronized with MRI acquisition.

General magnetic resonance imaging procedures

All MRI experiments were performed on a 9.4-T/31-cm MR system interfaced by a DirectDrive console (Agilent Tech, Santa Clara, CA) and an actively shielded gradient coil with 40 G/cm peak gradient strength and 120 μs rise time (Magnex, UK). The head of the rat was fixed in a non-magnetic head restraint with a bite bar and ear plugs. A home-built 1-cm inner diameter surface coil was positioned dorsal to the olfactory bulb for radio-frequency excitation and reception (see Fig. 1B). Preliminary spin-echo images of the entire bulb were acquired and used to select five 0.5-mm thick slices without gap for subsequent imaging studies with a 7×7-mm^2 FOV (see images in Fig. 1B). Both removal of the dorsal scalp and the diminished coil sensitivity in the ventral bulb minimized folding artifacts in the dorsal–ventral phase-encoding direction.

High resolution anatomical and blood volume images

High resolution anatomical, T_2* and T_2-weighted images were acquired with a matrix size of 128×128 (55×55 μm^2 in-plane resolution). Anatomical images were acquired with a fast spin-echo sequence in all six animals and imaging parameters of 5.0 s T_R, train of 4 echoes, 40.7 ms effective T_E and 24 averages. T_2^*-weighted images were acquired with a fast low-angle shot (FLASH) sequence with a 190 ms T_R, 6.0 ms T_E and 40 averages. T_2-weighted images were acquired with a multi-echo, spin-echo sequence with a 3.0 s T_R, train of 8 echoes, T_E = 12.5, 25.1, 37.6, 50.1, 62.7, 75.2, 87.8 and 100.3 ms, and 10 averages. The high-resolution T_2^* and T_2-weighted images were acquired before and approximately 5 and 150 min after MION injection (15 mg/kg) in four of the six animals, respectively.

fMRI data acquisition

The odor-evoked BOLD fMRI methods were adapted from previous studies in the olfactory bulb (Kida et al., 2002; Xu et al., 2000; Yang et al., 1998), followed by CBVw fMRI. The FLASH sequence was used to obtain high-quality images in and around large magnetic susceptibility areas surrounding the sinuses, where the echo planar imaging technique induces large image distortions. Imaging parameters were an